IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Wilson *et al*

Serial No.: 10/575,112

Filed: October 8, 2004

For: USE OF PHOTOSENSITISATION

DECLARATION

I, Michael Wilson, do hereby declare and state as follows:

1. I am a Professor of Microbiology at the Eastman Dental Institute, University College London. My main research interests are indigenous microbiota of humans, antibiotic resistance, biofilms, bacterial virulence factors, and the development of new antimicrobial strategies, in particular light-activated antimicrobial agents and their application to the treatment and prevention of infectious diseases. A selection of recent publications on which I am named as an author is attached as Annex I.

2. I am one of the inventors named on US patent application no. 10/575,112.

3. I have read and understood the Office Action from the US Patent and Trademark Office dated December 8 2010. I have also read and understood Hogset (WO 02/44395) and Norris (US 2004/0220123). I am a named author of Embleton *et al.* (*J. Antimicrob. Chemoth.* (2002) **50**, 857-864).

4. I have been asked to comment on (a) the unexpected properties of the present invention compared with the prior art, in particular Embleton, and (b) whether or not those properties would have been predictable from Hogset, Embleton and Norris.

(a) **Unexpected properties**

5. Embleton presents results of lethal photosensitization of methicillin resistant *Staphylococcus aureus* (EMRSA-16) using an antibody-photosensitiser conjugate, specifically IgG-SnCe6 (tin chlorin). Figure 3(b) of Embleton shows that a 2 log reduction (99% kill) in the viable count of EMRSA-16 was achieved using a

photosensitizer concentration of 3μg/ml at a light dose of 21 J/cm^2.

6. We found that a bacteriophage-photosensitiser conjugate, comprising staphylococcal phage 75 and tin chlorin e6 photosensitiser, was surprisingly more effective than the antibody-photosensitiser conjugate of Embleton, when the same photosensitiser (tin chlorin) was used. Thus Example 1 of the present application shows that a 4 log reduction (99.99% kill) in the viable count of EMRSA-16 was achieved using a bacteriophage-tin chlorin conjugate with half the photosensitiser concentration, at the same light dose (see Figure 1).

7. Further experiments using a bacteriophage-photosensitiser conjugate according to the present invention are reported in "Development of a novel targeting system for lethal photosensitization of antibiotic-resistant strains of *Staphylococcus aureus*" Embleton ML, Nair SP, Heywood W, Menon DC, Cookson BD, Wilson M. *Antimicrob Agents Chemother.* 2005;49:3690-6, a copy of which is attached. Figure 2 of this paper shows that the bacteriophage-tin chlorin conjugate at the same photosensitiser concentration as Figure 3(b) of Embleton of 3μg/ml and the same light dose achieved a 4 log reduction (99.99% kill) in the viable count of EMRSA-16.

8. We also found that the bacteriophage- photosensitiser conjugate was able to achieve a similar degree of killing of the EMRSA-16 at all three growth phases (see Figure 3 of the *Antimicrob Agents Chemother.* 2005 paper). This is in contrast to the antibody-photosensitiser, as shown in Figure 4 of Embleton, in which killing was markedly affected by the growth phase. Thus in Embleton, killing of the lag-phase bacteria was considerably reduced. This is particularly surprising as the concentration of bacteriophage binding sites on the bacterial cell is known to be affected by the growth phase of the organism. Consequently it would have been expected that bacteriophage-mediated killing would be affected by growth phase. Surprisingly, this was not the case.

9. One of the features common to vancomycin-intermediate strains of *S. aureus* is thickening of the cell wall in the presence of the antibiotic. Cell wall thickening may result in reduced susceptibility of the organism to lethal photosensitization for a number of reasons. First, the major targets of singlet oxygen are the bacterial cell wall and membrane, so that any increase in the thickness of the cell wall could protect both structures against the harmful effects of singlet oxygen. Second, it may alter the number and/or distribution of bacteriophage receptors and so could affect the number of molecules of the phage-photosensitiser conjugate adsorbed to the bacterial surface. We found that after growth in up to 16 mg/liter of vancomycin, strains Mu3 and Mu50 (which are vancomycin-intermediate) remained highly susceptible to lethal photosensitization using the bacteriophage- photosensitiser conjugate. This is shown in Figure 5 of the *Antimicrob Agents Chemother.* 2005 paper.

Thus, surprisingly, the bacteriophage-photosensitiser conjugate was effective even in the presence of antibiotic.

10. I would expect these findings of (i) improved killing over an antibody-photosensitiser conjugate, (ii) killing of bacteria in all three growth phases and (iii) killing in the presence of an antibiotic to be generally applicable to other combinations of staphylococcal bacteriophage and photosensitiser selected from chlorins and phenothiaziniums because the nature of the photosensitiser is unlikely to affect those properties of the photosensitiser-phage conjugate that are relevant to these activities.

(b) **Results not predictable from Hogset, Embleton and Norris**

11. Having read and considered Hogest, Embleton and Norris, I cannot find anything which suggests to me that conjugating a staphylococcal bacteriophage to a photosensitiser selected from chlorins and phenothiaziniums via a covalent bond could result in a composition with the improved antibacterial properties described above.

12. In particular, Hogset is concerned with targeting eukaryotic cells, and therefore does not seem to me to be relevant to consideration of killing bacteria, which are prokaryotic cells. Embleton is concerned with killing bacteria (i.e. prokaryotic cells). I would not therefore have read these documents together when considering the provision of improved antibacterial compositions. Furthemore, Norris is concerned with the delivery of toxic gene products, and is not concerned with photodynamic therapy, and so I would not have read this document together with Hogset and Embleton. In my view, the results on which the present invention is based would not have been predictable from Hogest, Embleton and/or Norris.

13. I acknowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, and may jeopardize the validity of the application or any patent issuing thereon. All statements made of my own knowledge are true and all statements made on information and belief are believed to be true.

Signed 

This 28 Day of March 2011.

ANNEX I

Recent Papers

Antimicrobial properties of light-activated polyurethane containing indocyanine green
Perni, S, Pratten, J, Wilson, M, Piccirillo, C, Parkin, I P, Prokopovich, P
J Biomater Appl 2011: 25(5), 387-400

Antimicrobial Activity in Thin Films of Pseudobrookite-Structured Titanium Oxynitride under UV Irradiation Observed for Escherichia coli
Aiken, ZA, Hyett, G, Dunnill, CW, Wilson, M, Pratten, J, Parkin, IP
CHEM VAPOR DEPOS 2010: 16(1-3), 19

Effective photoinactivation of Gram-positive and Gram-negative bacterial strains using an HIV-1 Tat peptide-porphyrin conjugate
Bourré, L, Giuntini, F, Eggleston, IM, Mosse, CA, Macrobert, AJ, Wilson, M
Photochem Photobiol Sci 2010: 9(12), 1613-1620

Antibacterial Activity of Light-Activated Silicone Containing Methylene Blue and Gold Nanoparticles of Different Sizes
Perni, S, Piccirillo, C, Kafizas, A, Uppal, M, Pratten, J, Wilson, M, Parkin, IP
J CLUST SCI 2010: 21(3), 427-438

The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles
Perni S, Piccirillo C, Pratten J, Prokopovich P, Chrzanowski W, Parkin IP, Wilson M
Biomaterials 2009: 30; 89-93

***In vivo* killing of *Staphylococcus aureus* using a light-activated antimicrobial agent**
Zolfaghari PS, Packer S, Singer M, Nair SP, Bennett J, Street C, Wilson M
BMC Microbiology 2009; February 4th; 9: 27

The inability of a bacteriophage to infect *Staphylococcus aureus* does not prevent it from specifically delivering a photosensitiser to the bacterium enabling its lethal photosensitisation
Hope C, Packer S, Wilson M, Nair S
J Antimicrob Chemother 2009; 64: 59-61

Susceptibility of MRSA biofilms to denture-cleansing agents
Lee D, Howlett J, Pratten J, Mordan N, McDonald A, Wilson M, Ready D.
FEMS Microbiol Lett. 2009; 291: 241-6

Toluidine blue-containing polymers exhibit potent bactericidal activity when irradiated with red laser light
Perni S, Prokopovich P, Piccirillo C, Pratten J, Parkin IP, Wilson M.
J Mater Chem 2009; 19; 2715-23

Distribution of tetracycline and erythromycin resistance genes among human oral and fecal metagenomic DNA
Seville LA, Patterson AJ, Scott KP, Mullany P, Quail MA, Parkhill J, Ready D, Wilson M, Spratt D, Roberts AP.
Microb Drug Resist. 2009; 15: 159-66.

Antimicrobial surfaces and their potential in reducing the role of the inanimate environment in the incidence of hospital-acquired infections
Page K, Wilson M, Parkin IP
J. Mater. Chem., 2009; 19; 3819 – 3831

Antimicrobial activity of methylene blue and toluidine blue O covalently bound to a modified silicone polymer surface
Piccirillo C, Perni S, Gil-Thomas J, Prokopovich P, Wilson M, Pratten J, Parkin IP.
J. Mater. Chem. 2009; 19; 6167-71

Improved peptide prodrugs of 5-ALA for PDT: rationalization of cellular accumulation and protoporphyrin IX production by direct determination of cellular prodrug uptake and prodrug metabolization
Giuntini F, Bourre L, MacRobert AJ, Wilson M, Eggleston IM
J. Medicinal Chem 2009; 52; 4026-4037

White light induced photocatalytic activity of sulfur-doped TiO2 thin films and their potential for antibacterial application
Dunnill CW, Aiken ZA, Kafizas A, Pratten J, Wilson M, Morgan DJ, Parkin IP
J. Mater. Chem. 2009; 19; 8747–8754

Frictional properties of light-activated antimicrobial polymers in blood vessels
Prokopovich P, Perni S, Piccirillo C, Pratten J, Parkin IP, Wilson M
J Mater Sci: Mater Med 2009; DOI 10.1007/s10856-009-3882-2

Inactivation of staphylococcal virulence factors using a light-activated antimicrobial agent
Tubby S, Wilson M, Nair SP
BMC Microbiology 2009; 9: 211